Cell death: **TRAIL and its receptors**

Pierre Golstein

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The recently booming field of cell death research has been focusing in particular on signaling, especially via cell-surface receptors of the tumor necrosis factor receptor (TNFR) family. Some of these receptors are homologous to each other not only in extracellular cysteine-rich domains, but also in a cytoplasmic 'death domain' [1,2], a protein–protein interaction module that ensures downstream cell-death signaling. The receptor family includes the well studied Fas/Apo1 (CD95) and TNFR1 molecules. Engagement of the receptors by Fas ligand (Fas-L) or by TNF (or lymphotoxin), respectively, can lead to cell death [3–6]. These ligands and receptors were the first known members of a multilevel molecular system connecting extracellular signals and the intracellular cell-death cascade. Figure 1 is a composite representation of most of the currently known molecules operating at each of these levels in various cells: ligands, cell-surface receptors, adaptors (chimeric molecules bearing modules that enable them to interact with both receptors and effectors), and effectors (bearing 'caspase' protease modules, the activation of which triggers a caspase cascade that is most often required for cell death) [4–6].

New molecules have recently been added to the cell-surface receptor level of this multilevel system. CAR1 was identified [7] as a chicken cell-surface receptor for cytopathic avian leukosis sarcoma viruses. It shows homology to TNFR1 family receptors: it contains two extracellular cysteine-rich domains and a death domain. In CAR1-transfected cells, CAR1 was shown to be bound by a viral envelope (Env) fusion protein, and binding led to cell death. The physiological ligand of CAR1 is not yet known [7]. This shows that, like many other surface molecules, members of the TNFR1 family can be borrowed by viruses as entry points, although it is not completely clear what advantage is conferred to the virus by thus running the risk of inducing the death of the cell it is infecting. Another receptor, mammalian death receptor 3 (DR3) [8] (also called WSL-1 [9], Apo3 [10], LARD [11], and TRAMP [12]), shows the highest sequence similarity to TNFR1; it contains four extracellular cysteine-rich domains and a death domain. Overexpression of DR3 leads to cell death. Given its abundant expression on thymocytes and lymphocytes, it would not be surprising to find that DR3 plays a significant role within the immune system, but no information has yet been forthcoming on this issue. Moreover, as a current major 'black box' in this field, and perhaps reminiscent of early days in the analysis of the Fas system, the ligand for DR3 is not yet known.

Ironically, the current flurry of activity regarding new receptors stemmed from the discovery of a novel ligand that bound neither DR3, CAR1, nor any of the other previously known members of the TNFR1 family. This ligand was called TRAIL (for TNF-related apoptosis-inducing ligand [13]) or Apo2-L [14]. Data from two research groups [13,14] indicated that TRAIL is a type II membrane protein, the carboxy-terminal extracellular domain of which shows clear homology to other TNF family members. In contrast to some TNF family members, the amino-terminal intracellular domain of TRAIL is short and not conserved between mouse and man, making it unlikely that this domain itself would have a function in the TRAIL-bearing cell. Engineered soluble TRAIL molecules seem to be multimeric in solution. The TRAIL gene is located on chromosome 3 at position 3q26, which is not close to genes encoding other known TNF family members [13] nor to genes encoding the TRAIL receptors (see below).

Early results from TRAIL analyses immediately raised a problem. On the one hand, expression of TRAIL, at least as RNA transcripts, was detected in many human tissues, particularly in spleen, thymus, prostate and lung, but not in brain, liver, testis, unstimulated or stimulated peripheral blood T cells. On the other hand, while many human lymphoid as well as non-lymphoid tumor cell lines were sensitive to cell-surface or soluble TRAIL [14], normal cells, such as freshly isolated mouse thymocytes or primary B or T cells, were not. This led to the early remark that "Given the rather widespread expression of TRAIL and its ability to induce apoptosis in so many different cell types of cultured cells, it is reasonable to infer that either the TRAIL receptor is restricted in its distribution, or that it acts to induce apoptosis only under certain restricted circumstances" [13]. Indeed, at this point a reasonable assumption was that TRAIL, a widespread and apparently constitutive ligand, must have a more restricted and inducible receptor, the expression of which

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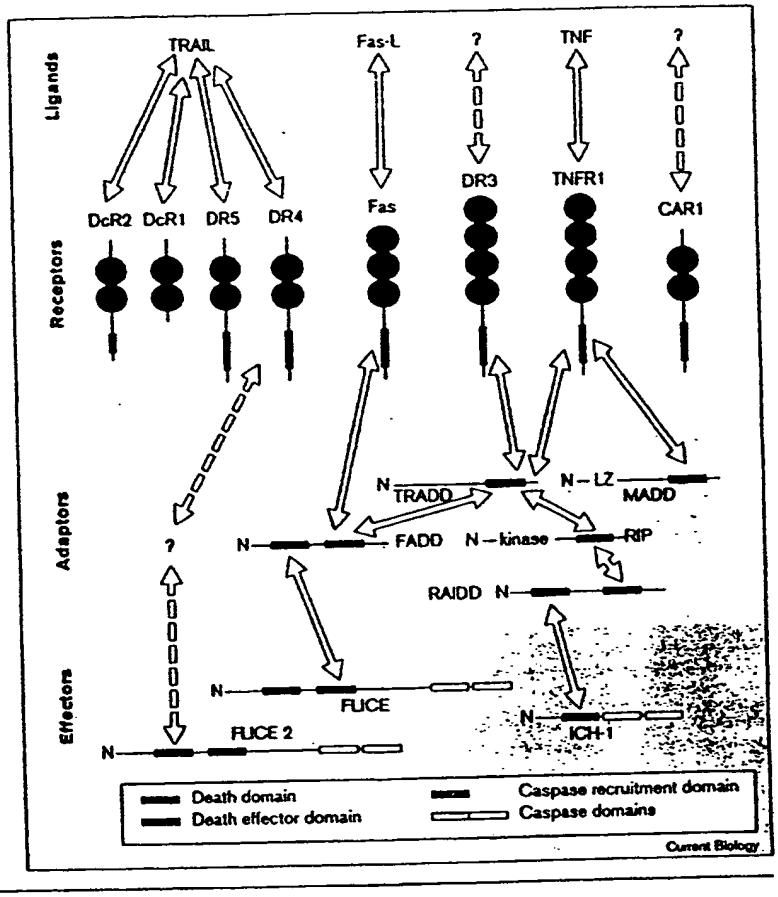
Figure 1

A multilevel cell-death signaling system linking ligands (either on the same cell, *in cis*, or on another cell or in solution, *in trans*), via cell-surface receptors and adaptors, to effector molecules with an activatable caspase domain. Receptors, adaptors and ligands interact via the indicated protein–protein interaction domains, between which there is some sequence homology [29]. The cell membrane, bearing the receptors, is not shown. Further details, references, and alternative names for the molecules shown are found in the text.



would regulate death. It now turns out, though, that nature found a different solution.

TRAIL did not languish very long waiting for a receptor; indeed, it soon got several. A first human receptor for TRAIL, named DR4, was found by searching an expressed sequence tag (EST) database for sequences with homology to the death domain of TNFR1 [15]. The extracellular region of DR4 contains two cysteine-rich domains and binds TRAIL specifically. It does not bind other known ligands of the same family, and TRAIL does not bind Fas or TNFR1. The cytoplasmic region of DR4 contains a death domain, and overexpression of DR4 leads to cell death, which does not occur if the death domain is deleted. DR4 is expressed, in terms of transcripts, in most human tissues, including spleen, thymus, peripheral blood leukocytes, activated T cells, small intestine, and some tumor cell lines. A second receptor for TRAIL, designated DR5 [16,17] (also called Trick2 [18], TRAIL-R2 [19] or KILLER/DR5 [20]), is quite similar in structure to DR4, with two cysteine-rich extracellular domains and a cytoplasmic death domain. DR5 triggers cell death when engaged, and it is expressed as transcripts in both normal tissues and cancer cell lines. In addition, expression is high in peripheral blood leukocytes and spleen and is upregulated upon lymphocyte activation [18].

Which pathways connect DR4 and DR5 to cell-death effectors (Figure 1)? Death induced by soluble TRAIL was not blocked by ectopic expression of a dominant-negative mutant of Fas-associated death domain protein (FADD), showing that at least one receptor for TRAIL uses a non-FADD pathway [21]. Indeed, DR4 was found not to bind to the known death-pathway adaptor molecules — FADD, TNFR-associated death domain protein (TRADD), receptor-interacting protein (RIP), or RIP-associated ICH-1/CED-3 homologous protein (RAIDD) — suggesting the exciting possibility that DR4 uses other proximal signaling machinery that has yet to be identified [15]. It is less clear whether DR5 interacts [19] or not [16]

with FADD: differences may be linked to the experimental systems used. Further downstream, for both DR4 and DR5, the protease effector molecule FADD-like interleukin-1β-converting enzyme-2 (FLICE2) seems to be at play [16], leading to caspase activation, which is required for cell death in these cases as well [15–17,19,21,22].

One would then like to understand how normal cells, which bear DR4 and/or DR5 and must encounter TRAIL-bearing cells, are not induced to die. How not to signal death? In this case, an answer was provided by the discovery of two other TRAIL receptors which, rather than transducing a death signal, seem to inhibit this transduction. Decoy receptor 1 (DcR1) [17] (also called TRID [16], LIT [23], or TRAIL-R3 [24]) has an extracellular TRAIL-binding region with two cysteine-rich domains and a transmembrane domain but, importantly, no intracellular signaling domain. In fact, DcR1 seems to be a cell-surface protein anchored by glycophosphatidylinositol (GPI) [17,24]. Other GPI-linked proteins have been reported to be involved in cell-death or cell-survival signaling [25,26]. Overexpression of DcR1 did not induce death and protected mammalian cells from TRAIL-induced cell death. DcR1 transcripts were detected in some normal human tissues, in particular in non-activated peripheral blood leukocytes and spleen, but not in most cancer cell lines examined [16].

Another receptor also apparently working as a decoy receptor has been described very recently and designated DcR2 [27] (also called TRUNDD; G. Pan, J. Ni, G. Yu, Y. Wei, V.M. Dixit, personal communication). DcR2 is homologous to other TRAIL receptors and bears two extracellular cysteine-rich domains able to bind TRAIL. The cytoplasmic region of DcR2, however, bears a truncated death domain. DcR2 seems unable to transduce a death signal, but, presumably through its extracellular region, it blocks, in part, TRAIL-induced cell death. Corresponding transcripts were detected in many human tissues, particularly fetal liver and adult testis, but not in most cancer cell lines examined ([27]; G. Pan, J. Ni, G. Yu, Y. Wei, V.M. Dixit, personal communication). Thus, both DcR1 and DcR2 decoy receptors for TRAIL lack a functional death domain. Their recognition of TRAIL may well prevent TRAIL from engaging the functional TRAIL receptors, therefore, resulting in the blocking rather than the transduction of a death signal.

The surface decoy receptors DcR1 and DcR2 are thus implicated in the regulation of TRAIL cell-death signaling. Interestingly, the genes corresponding to these two regulatory receptors co-map, at 8p21, with the genes corresponding to the two signal-transducing receptors DR4 and DR5 [19,20,24,27]. Co-localization, together with structural and binding homology, suggests emergence of these four genes from a common precursor through recent duplications. The use of decoy receptors increases the diversity of known cell-death control mechanisms, such as differential inducible expression of ligand and/or receptor, production of soluble ligand and/or receptor, and differential expression or activation of various components of the intracellular death cascade.

Why tumor cells should be sensitive to TRAIL, as evidenced by TRAIL-induced death, is not clear, because one would expect resistance. A possible explanation might be that the TRAIL system exerts, on tumor cells as well as on normal cells, a main function other than cell death — such as the induction of survival or proliferation; other members of these families of molecules do have such functions. But TRAIL did not co-stimulate primary T cells to proliferate in the presence of suboptimal amounts of anti-CD3 antibodies [13] (data not shown). It could also be that decoy receptors are expressed in emerging primary tumors, making them resistant to TRAIL. Expression of these decoy receptors might decrease, and sensitivity to TRAIL might therefore increase, with time and/or size of the tumors, or long-term passage or culture (apparently it is mostly tumor cell lines, rather than primary tumors, that have been shown so far to express the decoy receptors poorly).

What could be the role(s) of the TRAIL receptors? They can transduce a signal interpreted as a cell-death signal, but under which physiological circumstances? Not much is known at present. Their tissue distribution is widespread, in particular (but not only) in lymphoid tissues, consistent with a role in the immune system. DR5 is upregulated upon lymphocyte activation, and TRAIL activated cell death in T-cell-enriched cultures of peripheral blood leukocytes stimulated by interleukin-2, but not in unstimulated cells [21]. This finding suggests that, like Fas ligand, TRAIL may play a role in regulating the death of activated lymphocytes [18,21]. Indeed, both Fas ligand and TRAIL contribute to activation-induced death of Jurkat cells (A. Anel and A. Ashkenazi, personal communication), and TRAIL contributes to activation-induced death of T cells in HIV infection [28]. Upon lymphocyte activation, DcR1 expression is lost, while expression of TRAIL is induced [23]. Thus, TRAIL increases, DR5 increases, DcR1 decreases and, perhaps correlatively, sensitivity to TRAIL increases upon activation. However, wide distribution of the TRAIL receptors outside the immune system leaves open the possibility of a non-immunological role of the TRAIL family. Interestingly, expression of DR5 is induced by p53 and by DNA-damaging agents via p53 [20], but it is not yet known whether this expression is required for p53-induced cell death.

TRAIL receptors, like several of the other receptors with death domains, are still in search of unambiguous functions in cell death. On the other hand, there are also still

cell-death situations in search of effector molecules, such as negative selection in the immune system and, outside the immune system, morphogenesis. Will the TRAIL system, with its unusual regulation, or perhaps other molecules of the same families of receptors and ligands, account for some of these phenomena?

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